Journal of Chemical and Natural Resources Engineering, Special Edition : 11-19

EVALUATION OF THE POTENTIAL OF *PELARGONIUM RADULA* EXTRACT IN REPELLING *AEDES AEGYPTI*

S. ASNAWI[1], Z. MOHD ZAKI[2], A. ABDUL AZIZ[1,2], A.K. KHAMIS,[1] B.ABDUL AZIZ[1]

ABSTRACT

Pelargonium radula (geranium) essential oil has been traditionally used to kill or repel mosquito. This study was performed to obtain information on the yield of geranium extract, its chemical composition and the potency of geranium extract. Geranium extracts were obtained using solvent extraction and steam distillation methods. Solvent extraction produced higher yield (7.94%) compared to steam distillation (2.54%). GC and GC/MS analysis of geranium revealed that its major constituent was geraniol (28.51%). Geranium oil was tested for their repellency activity against adult female mosquito dengue hemorrhagic fever, *Aedes aegypty*. Repellency test was evaluated using a repellency kit with humans as volunteers. Geranium oil extracted using steam distillation gave stronger activity with median effective concentration value (EC_{50}) of 0.0051 mg cm^{-2} compared to the geranium oil extracted using solvent extraction (EC_{50} of 0.0267 mg cm^{-2}).

Key Words: Repellency activity, *Aedes aegypti*, *Pelargonium radula* extract, active compounds, DEET

1.0 INTRODUCTION

In recent years, mosquito-borne diseases have become a major international public health concern. Many countries and areas in Asia as well as in Latin America have been experiencing unusually high levels of malaria, dengue, filariasis and Japanese encephalitis activity. These are the four important vectors borne disease in the world. The vectors responsible for the four diseases belong to the genera *Anopheles, Aedes, Mansonia* and *Culex*. These mosquitoes do not only transmit malaria, dengue, filariasis and viral disease but also cause allergic responses that include local skin reaction and systemic reaction. Fradin and Day [1] reported mosquitoes transmit diseases to more than 700 million persons from over 100 countries in a year. This includes the malaria disease which kills 3 million people each year, including one child every 30 seconds.

Plant essential oil, commonly used as a fragrance and flavoring agents for foods and beverages, are recommended as an alternative source for insect control. Generally, essential oil-derived compounds, which are classified as a plant secondary metabolite, with a few exceptions, can be applied to humans in a similar way to other conventional insecticides and they tend to be selective and have little or no harmful effect. *Pelargonium* spp. or geranium

[1]Bio-pestide Unit, Chemical Engineering Pilot Plant (CEPP), Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
[2] Department of Bioprocess Engineering, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
[3]Medical Plants Division, Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur.
Tel : +6075535525 Fax: +6075581463
Correspondence to : Azila Abdul Azi(azila@fkkksa.utm.my)

essential oil is a plant extract used as the active ingredient in insect repellent formulatio Another name for *Pelargonium radula* is *Pelargonium graveolens*. *Pelargonium radul* produces a characteristic essential oil having terpene alcohols as major components, e.g geraniol, cintronellol and their esters, e.g. i-methnone, citronellyl formate, and geran formate also eugenol [2]. These components are believed to have potential insect repellenc activity [3].

Conventionally, the essential oil of plants is isolated by hydrodistillation, stea distillation or solvent extraction [4]. The problems associated with this technique are lo yield, losses of volatile compound, long extraction time, degradation of unsaturate compounds and toxic solvent residue [5].

This work focused on the extraction of *Pelargonium radula* oil using differe extraction techniques, the analysis of the physico-chemicals characteristics of the oil and th determination of the effectiveness (EC_{50} and repellency, %) of *Pelargonium radula* extra on *Aedes aegypti*.

2.0 MATERIALS AND METHODS

2.1 Materials

Pelargonium radula plants were purchased from a nursery at Pulau Pinang. Durin the project, all samples were maintained in our lab at room condition. The standar compounds were purchase from MERCK, i.e., citronellol, geraniol and eugenol. Th compounds were then further diluted with ethanol (95% v/v) to the appropriate concentratio needed.

2.2 Methods

2.2.1 Extraction of Pelargonium radula

Steam distillation

The plant (100 g of dried leaves) in 500 ml flask was steam distilled for 8-10 h. The volat distilled was collected until no oil dropped out. The distilled was saturated with sodiu chloride and ether. Then, the ether layer and the hydro layer were separated using a funne After dehydrating using anhydrous sodium sulphate, the ether layer was further heated i 60°C water bath to concentrate the oil and recover the ether. The oil was weighed an refrigerated prior to analysis.

Solvent extraction

The extract of *Pelargonium radula* was obtained by solvent extraction (immersion method *Pelargonium radula* samples (30 g) were transferred into a beaker, then extracted with 30 ml absolute methanol (1:10) for 2 days. After that, extracts were concentrated using rota vacuum evaporator at 60 °C.

2.2.2 Bioactive Composition Confirmation

Thin layer chromatography

The steam distillation extracts of *Pelargonium radula* and standards (geraniol, citronellol, methanol, linalool, geranyl formate, citronellyl formate, geranyl acetate and eugenol) were dissolved in ethanol 95% (v/v). One microliter samples were applied to silica-gel TLC plate (G60F254, 20 cm x 20 cm, thickness 300μm, Merck®, Darmstadt, Germany) and developed with ethyl acetate: chloroform: methanol (9:1:01 v/v) . The detection was done by exposing the plates to sulfuric acid 10%, heating the plates to 120°C for 5–10 minutes and employing UV light at 245nm/365nm . The R^f was determined to identify the similarity of the spots with the standards.

Gas Chromatography, Gas Chromatography-Mass Spectrometer (GC, GC/MS) Analysis

GC analyses of the steam distillation extracts were performed employing a Perkin-Elmer gas chromatography (Model 8500) fitted with flame ionization detector (FID), GP-100 printer–plotter and an electronic integrator, using a bonded phase fused silica capillary column BP-1 (25 m length x 0.5 mm i.d.; film thickness 0.25 μm) coated with polydimethylsilixone. Nitrogen at a flow rate of 40 ml/min (linear velocity 34 cm/s) and 10 psi inlet pressure was the carrier gas employed. The analysis temperature was from 60 to 220°C at 5°C/min ramp rate with a final hold time of 10 min. Injector and detector were maintained at 250 and 300°C, respectively. The extracts (0.1-0.2 μl) were injected neat with 1:80 split ratios.

GC/MS analyses of extracts from steam distillation was performed using a Hewlett-Packard 5890 gas chromatography coupled to a HP 5970 mass-selective detector (MSD) using a fused silica ultra performance cross-linked methyl silicone column (50 m length x 0.2 mm i.d.; film thickness 0.25 μm). Temperature programming was done from 100 to 280°C at 4°C/min. Helium was used as the carrier gas at 1 min/min flow rate. Mass spectra were recorded over 40-400 amu range at 1 scan/s with ionization energy 70eV and ion source temperature of 250 °C.

Extract component was identified from the retention time of the chromatogram peaks compared to those of reference compounds run under identical conditions. Retention indices were compared. Retention indices were computed from gas chromatograms by logarithmic interpolation between n-alkanes.

2.2.3 Repellency Assessment

Mosquito repellency activity was assessed using the test cage described in the American Society for Testing and Material (ASTM) Standard E951-83 for laboratory testing of noncommercial mosquito repellent formulations on the skin [6] with slight modification. The test procedures were similar to that described by previous researchers. The flexor regions of the forearms of volunteers were outlined with five circular 29 mm diameter test areas. A volume of 0.025 ml of series dilutions of the extract of *Pelargonium radula* in ethanol (0.0006 – 0.0379 mg cm^{-2}) and 0.025 ml of the diluents were applied randomly to the marked areas of first, second, fourth and fifth circles. Ethanol 95% was applied at the middle, which was the third circle as the control test. DEET (diethyl-m-toluamide) was used as a standard

repellent. The test cages were positioned securely on the arms of each volunteer with Vel tapes to ensure that only test areas were exposed to mosquito bites. Fifteen fem mosquitoes, three to five days old were introduced into each cage and the number of bit was recorded at the end of 120 sec. The test procedure was replicated three times for each sample and statistically reliable estimates of their median effective concentrations (EC were obtained by probit analysis program. Percentage repellency was determined by following formula [7]:

$$\text{Repellency} = \left[\frac{100 - T}{C} \right] \quad x \quad 100 \qquad (1$$

where T is the total number of bites on treatment area and C is the total number of bites control area.

2.2.4 Probit Analysis

All data obtained were subjected to log-probit analysis[8]. Data were keyed in into program to obtain their effectiveness concentration values (EC) from 1 to 99 (EC_1 to EC These computer generated program would provide the LC and EC values with appropri regression lines and slopes, their range of confidence interval at 95% confidence lir variance of the EC_{50} values and the heterogeneity of the test. For the result purposes, E and EC_{90} would be chosen as comparison values of the activity.

3.0 RESULTS AND DISCUSSION

3.1 Process Yield and Chemical Characterization

The yield obtained using solvent extraction method for *Pelargonium radula* was 7.94% 30g. The yield was higher than the yield for water distillation extraction method, which v 2.54% of oil per 100g of dried sample (Table 1). The oil isolated from the green foliage a sometimes also from the flowers, by hydrodistillation or steam distillation, was greeni yellow color with a rose mint odor. The odor of the plant changes from lemon-like to rc like when the leaves turn from green to yellow [9].

Table 1 Characteristics of the geranium extract

Geranium extract	Yield (%)	Extraction period (h)	Color and texture
Solvent extraction	7.94	48	Brown ointment
Steam distillation	2.54	8-10	Pale yellow oil

This yield obtained was significantly less than the yield obtained by Roa *et al.* [which was 92.3% per 100g. Yield can be affected by the spacing of the plant. Plant spac of around 60 cm can produce oil yield of 17.3% compared with plant spacing of around cm (7.9%) [11]. The condition of the plant whether fresh/dried and green/yellow will a affect yield. In one study, the oil yield from fresh plant was 0.1-0.2% only [9].

Wenqiang *et al.* [12] reported that extracts using solvent extraction method will produce lowest main active ingredients although its yield is the highest among other extraction methods. In addition, extracts obtained using the solvent method will be brown ointment, which means more undesired impurities and organic solvent residue may exist. Some studies suggest using supercritical CO_2 (SFE) to extract essential oil, because it offers the most important advantages over other methods such higher extraction yield, higher percentage of active ingredients and shorter extraction time [4].

3.2 Bioactive Composition

Thin layer chromatography of *Pelargonium radula* extract obtained from steam distillation gave rise to spots with R_f's similar to those of citronellol, geraniol, linalool, methone and geranyl acetate, but did not reveal spots with R's similar to that of geranyl formate, citronellyl formate and eugenol. The results of analysis of *Pelargonium radula* oil by combined gas chromatography and mass spectrometry are shown in Table 2. The most abundant constituent (28.51%) of *Pelargonium radula* oil, in terms of relative percentage of total volatile oil area in the chromatogram, was geraniol (Table 2) followed by geranyl acetate (27.63%), menthone (20.78%), linalool (3.75%) and citronellol (1.33%), respectively. There are two unidentified compounds.

Table 2 Chemical composition of the geranium oil obtained from the leaves of *Pelargonium radula* as determined by gas chromatography and mass spectrometry (GC/MS)

Peak	Oil constituent	Retention time (min)	Area (%)[a]
1	NI	10.08	10.98
2	Linalool	10.84	3.75
3	Methone	11.33	20.78
4	Citronellol	12.24	1.33
5	Geraniol	12.52	28.51
6	Geranyl acetate	13.65	27.63
7	NI	14.72	7.08

NI: not identified

[a] Relative percentage of total volatile oil

The foregoing finding thus indicated that eugenol, methone and citronellol, which together amounted to 50.62% of the total oil, were the major constituents of the *Pelargonium radula* essential oil evaluated in the present study. Zaki *et al.* [13] reported that the oil of *Pelargonium* sp. has 40,73% geraniol and 11.96% β-citronellol. Balchin [14] reported that the plant has 41.9% geraniol and 17.6% citronellol. The plant's essential oil has a sweet rosy and lemon aroma which comes mainly from the high content of geraniol.

The chemical composition of essential oil of *Pelargonium* spp. shows a large interspecies variability, and within the same species, it seems to depend on the genetic characteristics of the plant and the conditions under which it was grown [14]. Balchin [14] compared chemical compounds of essential oil between Indian geranium and Iranian geranium oil, and found that the Iranian oil is richer in citronellol and citronellyl formate compared to the Indian oils. However, the Indian geranium oil is richer in geraniol and

citronellol compared to Iranian oil. Variation in the percentage composition of the m constituents may be due to the variation in their agroclimatic and geographical conditio the time harvested, fertilizers applied and others [15].

3.3 Repellency Test

Table 3 shows the percentage repellency of the *Pelargoium radula* extract towa *Aedes aegypti* at various concentrations. The leaf extract showed significant degree repellency. However, they displayed lower repellency efficiency compared to the stand repellent, DEET. The essential oil of *Pelargonium radula* and ointment oil of *Pelargoni radula* at 0.1514 mg cm^{-2} concentration provided 83.3 and 72.7% protection agai mosquito bites, respectively. Table 4 shows that the essential oil of *Pelargonium radula* l some degree of repellency activities with median effective concentration (EC_{50}) ranging fr 0.0051 to 0.0267 mg cm^{-2}. DEET with EC_{50} 0.0005 mg cm^{-2} was the most effective. Mio *al.* [16] compared andiroba oil (*Carapa guianensia*) with DEET. DEET was found perform better than andiroba oil. *Pelargonium radula* extracted using steam distillat produced volatile oil compared to *Pelargonium radula* extracted using solvent, wh produced ointment. Volatile oil has stronger aroma compared to ointment oil and it belie that this aroma come from the geraniol compound. This is another reason while the vola oil is more effective and needed lower concentration to repel mosquito compared to ointment oil (EC_{50} of 0.0051 mg cm^{-2} compared to 0.0267 mg cm^{-2}). This plant showed l potency in repelling mosquitoes compared to the citronella oil of *Cymbopogon nardus* (s wangi) which has the EC_{50} value of 0.0009 mg cm^{-2} [15,17].

Table 3 Percentage repellency of ointment oil of *Pelargonium radula*, essential oil *Pelargonium radula* and DEET toward *Aedes aegypti*

Concentration mg cm^{-2}		**0.1514**	**0.0757**	**0.0379**	**0.0189**	**0.0095**	**C**
Percent Repellency (%)	Ointment oil (Solvent extract)	72.7	60.0	54.5	45.5	0	
	Essential oil (steam distillation)	83.3	75.0	54.5	54.5	0	
	DEET	100	100	83.3	50	33.3	

Table 4 Effective dose of repellency activity of *Pelargonium radula* extract against adu mosquito of *Aedes aegypti* L.

Geranium extraction	Effective concentration	Value (mg cm^{-2})	95% CL	Regression Li (Slope ± SE
Solvent extract **(ointment oil)**	EC_{50}	0.0267	0.0123 – 0.0407	0.796 ± 0.20
Steam distillation **(essential oil)**	EC_{50}	0.0051	0.0012 – 0.0088	0.710 ± 0.20
DEET	EC_{50}	0.0005	0.0027 – 0.0009	2.750 ± 0.54

Note: EC_{50} = Median effective concentration; CL= confidence interval at 95% confidence level; SE=standard error.

This work shows that the extract of *Pelargonium radula* has potential repellency activity, but it needs high concentration to be more effective that the standard repellent, DEET. This plant contain active compound such as geraniol and citronellol, which have been shown to possess repellency activity [17]. Pure citronellol gave the strongest activity with median effective concentration value (EC_{50}) of 0.00011 mg cm^{-2} followed by pure geraniol (0.00018 mg cm^{-2}). In another study, scientists suggested that vanillin mixed with the essential oil could improve the repellency efficacy and new technologies to prolong the effectiveness of the essential oil such as encapsulation in solid lipid nanoparticles can also improve its potency [18].

4.0 CONCLUSIONS

In conclusion, the plant of *Pelargonium radula* was found to have some repellency activity in controlling mosquito. Compared to ointment oil of *Pelargonium radula,* its essential oil obtained from steam distillation has better mosquito repellency activity. This is because the essential oil have a sweet rosy and strong aroma which indicated higher concentration of geroniol.. Both of these oils have the potential to be developed as botanical pesticide but its performance under field condition needs to be determined.

ACKNOWLEDGMENT

The authors are gratefully thanked Head of Medical Entomology Department, IMR and Mr. Ezhar Abas in supplying the mosquito eggs sample and staff of Medical Plants Division, FRIM for their technical support. This project was funded under eScienceFund (project no: 02-01-06-SF0075), Ministry of Science, Technology and Innovation, Malaysia.

REFERENCES

[1] Fradin, S. M. and J. F. Day. 2002. Comparative Efficacy of Insect Repellents against Mosquito Bites. *New Engl Journal Medicine*. 1: 47-49.

[2] Kalodjera, Z., N. Blazevic and M. Volenec. 2001. Composition of the Essential Oil of Pelargonium radula (Cav.) L'Herit: A Study of its Vegetative Cycle. *Acta Pharm.* 51: 153-157.

[3] James, K. S. 2003. *Pelargonium* species. In: Balchin, M. L. Geraniums or Pelargonium: *The Genera Geranium and Pelargonium*. New Fetter Lane, London: Taylor and Francis. 11-15

[4] Ghasemi, E., Y. Yamini, N. Bahramifar and F. Sefidkon. 2007. Comparative Analysis of the Oil and Supercritical CO2 Extract of Artemisia sieberi. *Journal of Food Engineering*. 79: 306-311.

[5] Tam, C. U., F. Q. Yang, Q. W. Zhang, J. Guan and S. P. Li. 2007. Optimization Comparison of Three Methods for Extraction of Volatile Compounds from Cyp rotundus Evaluated by Gas Chromatography-Mass Spectrometry. *Journal Pharmaceutical and Biomedical Analysis*. 44: 444-449.

[6] Am. Soc. For Testing and Materials..1983. *Annual Book of ASTM Standa* Philadelphia.. 9: 51-83

[7] Weaving, A. J. S. and N. K. Sylvester. 1967. Pyrethrum as an Insect Repellent, II: A Laboratory Technique for its Evaluation as a Mosquito Repellent and Influence of Formulation on Persistence. *Pyrethrum Post*. 9: 31-35.

[8] Raymond, R. 1985. Log-Probit Analysis Basic Programme of Microcompu *Cahiers ORSTOM Serie Entomologie Parasitologie*. 23: 117-121.

[9] Gomes, P. B., V. G. Mata and A. E. Rodrigues. 2004. Characterization of Portugu Grown Geraniu Oil (*Pelargonium* sp.). *Journal of Essential Oil Research*. 16(5): 2 228.

[10] Roa, R. B. R., P. N. Kaul, K. V. Syamasundar and S. Ramesh. 2002. Water Solu Fraction of Rose-Scented Geranium (*Pelargonium* species) Essential oil. *Bioresou Technology*. 84: 243-246.

[11] Roa, R. B. R. 2002. Biomass Yield, Essential Oil Yield and Essential Composition of Rose-Scented Geranium (*Pelargonium* species) as Influenced Row Spacing's and Intercropping with Cornmint (*Meintha arvensis* L.f. *Piperas maalinv*. Ex Holmes). Industrial Crops and Product: *An International Journal*. 133-144.

[12] Wenqiang, G., L. Shufen, Y. Ruixiang, T. Shaokun and Q. Can. 2007. Comparari of Essential Oils of Clove Buds Extracted with Supercritical Carbon Dioxide Other Traditional Extraction Methods. *Food Chemistry*. 101: 1558-1564.

[13] Zaki, Z. M., N. A. M. Ali, A. S. Ahmad, M. F. Zollpatah, H. Hassan, I. K. M Ja and S. A. A. Bakar. 2005. Four Monoterpene Compounds: Repellency Evalua Against Aedes aegypti. *Malaysian Journal of Science*. 24: 225-228.

[14] Balchin, M. L. 2002. Essential oils from difference Pelargonium species cultivars: their chemicals composition (using GC, GC/MS) and appearance trichomes (under EM). In: Balchin, M. L. *Geranium and Pelargonium: The ger Geranium and Pelargonium*. Londan & New York: Taylor & Francis. 147-165.

[15] Azah, N. M. A, M. Z. Zaridah, A. A. Said and M. Z. Faridz. 2003. Larvc Properties of Citronella (Cymbopogon nardus) Essential Oil from Two Differe Localities. *Tropical Biomedicine*. 20(2): 167-174.

[16] Miot, H. A., R. F. Batistella, B. K. de Almeida and D. E. C. Volpato. 2(Comparative Study of the Topical Effectiveness of the Andiroba Oil (*Car guianensis*) and DEET 50% as Repellent for *Aedes* sp. *Inst. Med. Trop. Sao Pa* 46(5): 253-6.

[17] Zaridah, M.Z., A. A. Said, N. M. A. Azah, M. Z. Faridz, S. A. B. Asha, and Rohani. 2005. Bioinsecticidal Activities and chemical Analysis of Pelargoni citrosum Essential Oil Against Aedes Aegypri Linneaus. Pp. 72-78. In: *Proceedin the Conference on Forestry and Forest Products Research*. Kuala Lumpur. Nov. 24.

[18] Tsuji, K. 1999. Application and Particle Design of Insecticide Microcapsules. In: Scher, H. B. *Controlled-Release Delivery System for Pesticide*. U.S: Marcel Dekker. 55-87.